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ORIGINAL ARTICLE

Y-STR haplotype diversity among the Khandayat population of Odisha, India

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Abstract We analyzed seventeen Y-chromosomal short tandem repeat (Y-STR) loci in a population sample of Khandayat community residing in Odisha, India in order to find out the haplotype diversity. Blood samples were collected from 136 unrelated male individuals and genomic DNA isolation was carried out by the standard organic extraction method followed by multiplex PCR amplification using an AmpF*l* STR Y-filer PCR amplification kit and genotyping. A total of 130 haplotypes were observed among the studied samples, out of which 126 were unique. Allele frequency and gene diversity were calculated. Haplotype diversity and discrimination capacity were found to be 0.999128 and 0.95588 respectively. Haplotypes of Khandayat population were compared with that of other Indian populations using AMOVA (analysis of molecular variance) tool to measure the genetic relatedness between various populations of India.

1. Introduction

Short tandem repeats (STRs) or microsatellites are highly polymorphic markers dispersed throughout the genome which make them suitable for studying genetic polymorphism. Y-chromosome is inherited patrilineally. Now-a-days, Y-chromosome specific short tandem repeats (Y-STRs) are extensively being used in forensic genetics due to its significant role in solving sexual assault cases, paternity testing, individual identification, and studying migration patterns.[1–8]

Indian sub-continent is inhabited by a very diverse population consisting of more than four thousand anthropologically defined population groups.[9–16] Population genetic studies based on DNA polymorphism have revealed the presence of a large extent of genetic variation among several Indian population groups.[17–21] Odisha is a state located on the southeast coastal region of India, which is inhabited by various population groups belonging to different strata of the hierarchical caste system. Khandayats belong to an ancient warrior group also known as Kshatriya and constitute over 35% of the state's population.[9] We analyzed DNA samples of the Khandayat community in order to find out the haplotype diversity using seventeen Y-STR loci. The Y-STR loci chosen for this study include: DYS 19, DYS 389I, DYS 389II, DYS 390, DYS 391, DYS 392, DYS 393, DYS 385a/b, DYS 437, DYS 438, DYS 439, DYS 448, DYS 456, DYS 458, DYS 635 and

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Y_GATA_H4. Haplotypes of the Khandayat population were compared with the haplotypes of 10 other Indian populations using AMOVA tool aiming to find out the genetic relatedness between various populations of India.[22]

2. Materials and methods

2.1. Samples

We collected blood samples using a standard procedure in 2 ml EDTA vacutainers (BD Biosciences, NJ, USA) from 136 healthy unrelated male individuals of the Khandayat Community along with proper consent and approval by the Ethics Committee. Blood samples were stored at 4 °C till further analysis.

2.2. DNA analysis

Genomic DNA extraction from blood samples was carried out by the standard organic method (Phenol–Chloroform extraction method).[23] The DNA samples were amplified via multiplex PCR using AmpF*l* STR Y filer PCR Amplification Kit™ (Applied Biosystems, Foster City, CA, USA) for 17 Y-STR loci. PCR amplification was carried out as per the manufacturer's instructions. The amplified DNA samples were analyzed on an ABI Prism 3130 *xl* Automated Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).[24] Allelic designations for different loci were obtained by GeneMapper ID software (v. 3.2).

2.3. Statistical analysis

Allele frequencies were calculated by direct counting. Gene diversity (GD) was calculated using the formula;[25]

$$GD = \frac{n}{n-1}\left[1 - \sum_{i=1}^{n} P_i^2\right]$$

where, P_i is the frequency of *i*the allele and n is the number of samples analyzed.

Haplotype diversity (HD) was calculated by the formula;

$$HD = \frac{n}{n-1}\left[1 - \sum_{i=1}^{n} X_i^2\right]$$

where, X_i represents haplotype frequency.

In order to find out the genetic relatedness between the Khandayat population of Odisha and other Indian population groups, we used online AMOVA (analysis of molecular variance) tool provided by the Y-chromosome haplotype reference database (YHRD, www.yhrd.org). A total of 11 population samples with 840 haplotypes were included in this study. The studied samples belong to following populations: Jharkhand, India (Sakaldwipi Brahmin) population sample with 65 haplotypes, Karnataka, India (Brahmin) population sample with 103 haplotypes, Kashmir, India (Saraswat Brahmin) population sample with 58 haplotypes, Maharashtra, India (Mahadev Koli) population sample with 65 haplotypes, Punjab, India (Balmiki) population sample with 62 haplotypes, Rajasthan, India (Saraswat Brahmin) population sample with 60 haplotypes, Southern India, India (Tamil) population sample with 126 haplotypes, Tamil Nadu, India (Iyengar) population sample with 67 haplotypes, Tripura, India (Tripuri) population sample with 65 haplotypes, West Bengal, India (Rajbanshi) population sample with 39 haplotypes, Odisha, India (Khandayat) population sample with 130 haplotypes. Population pairwise distances between the Khandayat population of Odisha and other Indian populations (Rst values) were calculated. Online AMOVA tool of YHRD was used for molecular variance analysis with 10,000 permutations for calculating P value.[26]

3. Results

We collected blood samples from 136 healthy unrelated male individuals of the Khandayat community of Odisha, India and analyzed them for 17 Y-STR loci in order to find out the haplotype diversity. A total of 130 different haplotypes were observed by combined analysis of 17 Y-STR loci in the studied samples. The observed haplotype details are discussed in the supplementary Table 1. 126 haplotypes were unique (96.923%), which were observed only once. Out of 130 haplotypes, 2 haplotypes were observed thrice (1.53%) and 2 haplotypes were observed twice (1.53%). Haplotype diversity (HD) value for the Khandayat population of Odisha was found to be 0.999128. Discrimination capacity (DC) value for the studied samples was calculated to be 0.95588.

Observed alleles for the 17 Y-STR loci along with their allelic frequencies are mentioned in Table 1. Gene diversity (GD) per locus ranged from 0.4223 to 0.9609 with an average GD value of 0.6892. The lowest gene diversity (0.4223) has been found at locus DYS391, wherein the most frequent allele has been allele 10 with a frequency of 74.26%. The highest gene diversity (0.9609) has been found in case of the bi-allelic marker DYS385a/b. The highest frequency shown by different alleles for each of the 17 Y-STR loci has been highlighted in Table 1.

For extensive analysis of the genetic relatedness, haplotypes of the Khandayat population of Odisha were compared via AMOVA with haplotypes of other populations of India. The details of Indian populations used for comparative analysis are discussed in Table 2. Analysis of molecular variance pairwise distances based on Rst values between the Khandayats and other Indian populations are described in Table 3. Results revealed that Khandayat population is not closely related to other Indian populations.

4. Discussion

Population genetic studies have revealed a large extent of genetic diversity among various Indian populations.[20] The present study was carried out with an aim to study the haplotypes diversity of the Khandayat population of Odisha at 17 Y-STR loci. Although data on various Indian populations have been reported, there are no published data available about the genetic structure of the Khandayat population of Odisha elucidating the haplotype diversity based on 17 Y-STR loci.[15–20] Among the 17 Y-STR loci analyzed, the highest gene diversity (0.9609) was observed for locus DYS 385a/b and the lowest gene diversity (0.4223) was observed in case of locus DYS 391 (Supplementary Table 1), which is in accordance with one

Table 1 Allele frequency and gene diversity values of 17 Y-STR loci in the Khandayat population of Odisha, India.

Alleles	Y-STR loci																
	DYS19	DYS 389I	DYS 389II	DYS 390	DYS 391	DYS 392	DYS 393	DYS 438	DYS 439	DYS 437	DYS 448	DYS 456	DYS 458	DYS 635	YGATA H4	Genotype	*DYS 385a/b
8								0.0294								10,18	0.0074
9					0.0588			0.2500								10,19	0.0074
10					**0.7426**	0.0368	0.0074	**0.3750**	0.2500						0.0588	10,20	0.0074
11		0.0074			0.1544	0.0662	0.1618	0.3456	**0.3456**						0.2721	11,16	0.0074
12	0.1544	0.2647			0.0441	**0.5441**	**0.3971**		0.2574						**0.4559**	11,17	0.0368
13	**0.3676**	**0.4632**				0.1250	0.3897		0.1103	0.0074		0.0074			0.2132	11,18	0.0294
14	0.1838	0.2647				0.2279	0.0441		0.0368	**0.5588**		0.0515	0.0294			11,19	**0.0662**
15	0.2353									0.3162		**0.5368**	0.1838			11,20	0.0368
16	0.0588									0.1103		0.3309	**0.2941**			11,21	0.0074
17										0.0074	0.0074	0.0735	0.2426			12,17	0.0588
18											0.1618		0.2206			12,18	0.0441
19											**0.5294**		0.0294			12,19	0.0147
20											0.2721					12,20	0.0294
21				0.2426							0.0147					13,16	0.0074
22				**0.3897**							0.0147					13,17	0.0735
23				0.1765												13,18	0.0588
24				0.1912												13,19	0.0809
25														0.0294		13,20	0.0515
26														0.1250		13,21	0.0221
27			0.3235											**0.2721**		14,14	0.0147
28			0.1765											0.1324		14,16	0.0074
29			0.1471											0.2500		14,17	0.0588
30			**0.3529**											0.1544		14,18	0.0221
														0.0368		14,19	0.0368
																14,20	0.0074
																15,17	0.0588
																15,18	0.0294
																15,19	0.0294
																15,20	0.0147
																15,21	0.0368
																16,17	0.0147
																16,20	0.0074
																18,19	0.0147
GD	0.7539	0.6500	0.7233	0.7269	0.4223	0.6353	0.6672	0.6816	0.7438	0.5797	0.6236	0.5987	0.7761	0.8102	0.6742		0.9609

GD = gene diversity value.

Haplotype diversity or HD = 0.999128.

* Table shows allele frequencies for each Y-STR locus except DYS385a/b for which genotype frequencies are reported. The genotype frequencies for DYS385a/b were calculated for the combination of two alleles.

Table 2 Details of studied Indian populations.

Sl. no.	Population name	Location	No. of haplotypes
1	Khandayat	Odisha	130
2	Sakaldwipi Brahmin	Jharkhand	65
3	Brahmin	Karnataka	103
4	Saraswat Brahmin	Kashmir	58
5	Mahadev Koli	Maharashtra	65
6	Balmiki	Punjab	62
7	Saraswat Brahmin	Rajasthan	60
8	Tamil	Southern India	126
9	Iyengar	Tamil Nadu	67
10	Tripuri	Tripura	65
11	Rajbanshi	West Bengal	39

Table 3 Analysis of molecular variance (AMOVA) pairwise distances based on Rst values between the Khandayat population of Odisha and other Indian populations.

Population	"JhBr"	"KBr"	"KSBr"	"MK"	"PB"	"RSBr"	"STm"	"TNI"	"TrI"	"WBRj"	"ODKh"
"JhBr"	–	0.0203	0.0000	0.0000	0.0000	0.0033	0.0030	0.0018	0.0000	0.0000	0.0000
"KBr"	0.0244	–	0.0037	0.0000	0.0000	0.0260	0.2305	0.0929	0.0000	0.0000	0.0000
"KSBr"	0.1021	0.0350	–	0.0000	0.0000	0.0001	0.0109	0.0406	0.0000	0.0000	0.0000
"Mk"	0.2019	0.1145	0.0661	–	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
"PB"	0.2854	0.1665	0.1632	0.1363	–	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
"RSBr"	0.0435	0.0203	0.0624	0.1395	0.2053	–	0.0113	0.0012	0.0000	0.0000	0.0000
"STm"	0.0373	0.0025	0.0235	0.0944	0.1481	0.0226	–	0.2469	0.0000	0.0000	0.0000
"TNI"	0.0544	0.0110	0.0197	0.0750	0.1526	0.0531	0.0028	–	0.0000	0.0000	0.0000
"TrI"	0.3140	0.2331	0.1934	0.2169	0.3374	0.2872	0.2028	0.2024	–	0.0002	0.0000
"WBRj"	0.3370	0.2743	0.2096	0.2485	0.3863	0.3129	0.2467	0.2362	0.0837	–	0.0000
"ODKh"	0.3486	0.2561	0.1620	0.1463	0.2431	0.3037	0.2120	0.2048	0.1232	0.1422	–

* Populations; JhBr-Jharkhand, India [Sakaldwipi Brahmin], KBr-Karnataka, India [Brahmin], KSBr-Kashmir, India [Saraswat Brahmin], MK-Maharashtra, India [Mahadev Koli], PB-Punjab, India [Balmiki], RSBr-Rajasthan, India [Saraswat Brahmin], STm-Southern India, India [Tamil], TNI-Tamil Nadu, India [Iyengar], TrI-Tripura, India [Tripuri], WBRj-West Bengal, India [Rajbanshi], ODKh-Odisha, India [Khandayat].

of the earlier findings in South Indian population data.[16] Haplotype diversity and discrimination capacity for the studied population were found to be 0.999128 and 0.95588 respectively, which imply that the 17 Y-STR loci studied in the Khandayat population are highly polymorphic. Comparative study of haplotypes of the Khandayat population with other Indian populations, suggests that Khandayat population is not closely related to other Indian populations.

5. Conclusion

A higher degree of haplotype diversity and discrimination capacity indicates that 17 Y-STR loci used in the current study are highly polymorphic among the Khandayat community. Thus, this set of Y-STRs can be used for forensic purposes like paternity testing, individual identification, genetic mapping etc. and this will add to the databank of various studies conducted on the Indian population as no previous Y-STR data is available in the literature for this population.

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None.

Conflict of interest

None declared.

Ethical approval

Necessary ethical approval was obtained from the institute ethics committee.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ejfs.2014.07.003.

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